

## Review

# Potential role of the lipoxygenase derived lipid mediators in atherosclerosis: leukotrienes, lipoxins and resolvins

**Martin Hersberger\***

Division of Clinical Chemistry and Biochemistry,  
University Children's Hospital Zurich and Center for  
Integrative Human Physiology, University of Zurich,  
Zurich, Switzerland

## Abstract

Atherogenesis is an inflammatory process with leukocytes infiltrating the arterial intima. The lipoxygenase pathways play a role in leukocyte recruitment through the generation of two classes of arachidonic acid lipid mediators, the leukotrienes and the lipoxins, and one class of omega-3 fatty acid metabolites, the resolvins. There is evidence from animal studies and human genetic studies that the leukotrienes and the enzymes necessary for their generation play a role in atherosclerosis, and possibly even in the development of the vulnerable plaque. Less is known about the effect of the anti-inflammatory lipid mediators in atherosclerosis, the lipoxins and the resolvins. Studies modulating the activity of an enzyme necessary for the production of these lipid mediators, 12/15-lipoxygenase, showed discrepant results in several animal models. Also, human genetic studies have not clearly dissected the effect of the enzyme on atherosclerosis. However, stable forms of the lipoxins and the resolvins protect animals from inflammatory diseases. Whether blocking the leukotrienes or applying anti-inflammatory lipoxins and resolvins will be effective in attenuating human atherosclerosis needs to be demonstrated in future studies. In this review, the biosynthesis of these lipid mediators, their biological effects and the evidence for their possible role in atherosclerosis are discussed with an emphasis on human disease.

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**Keywords:** atherosclerosis; inflammation; leukotrienes; lipoxins; lipoxygenase; resolvins.

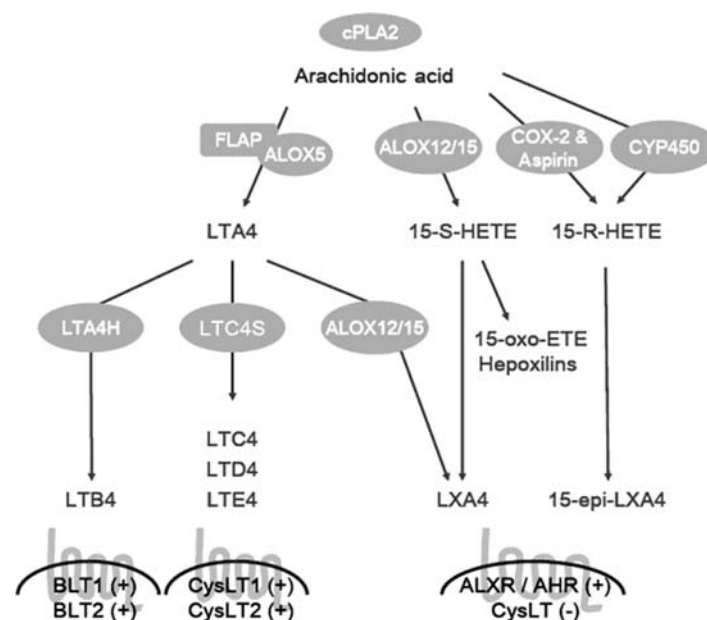
\*Corresponding author: Martin Hersberger, Division of Clinical Chemistry and Biochemistry, University Children's Hospital, Steinwiesstr. 75, 8032 Zurich, Switzerland  
Phone: +41 44 266 7541, Fax: +41 44 266 7169,  
E-mail: martin.hersberger@kispi.uzh.ch  
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## Lipid mediators in inflammation

When growth factors and cytokines bind to their receptors, they can activate several phospholipases which act on membrane phospholipids to release arachidonic acid (1). The released arachidonic acid forms a pool of precursors that are metabolized to distinct bioactive lipid mediators by a minor cytochrome P450 monooxygenase (CYP) pathway, and two major pathways: the cyclooxygenase (COX) pathway and the lipoxygenase pathway (2). The exact profile and balance of bioactive end products derived from this arachidonic acid pool depends on the type of cell and tissue, and is determined by the environmental and physiological context. While lipid mediators of the CYP pathway have vasoactive properties, and some of the genes of this pathway have recently been associated with coronary artery disease in genetic studies (3, 4), not much is known about their role in human atherosclerosis (5). In contrast, there is broad evidence for involvement of COX derived lipid mediators in the pathogenesis of atherosclerosis, and especially atherothrombosis, which has recently been summarized in two excellent reviews (6, 7). The third family of lipid mediators derives from arachidonic and from omega-3 fatty acids through oxygenation by lipoxygenases, and is the focus of this review.

## The lipoxygenase pathways in inflammation

The lipoxygenase pathway results in lipid mediators with opposing actions on inflammation. The pro-inflammatory leukotrienes and the anti-inflammatory lipoxins, resolvins and protectins show sequential evolution during acute inflammation (Figure 1). The pro-inflammatory leukotrienes are secreted following the appearance of polymorphonuclear neutrophils (PMN) in the acutely inflamed region, while anti-inflammatory lipoxins and resolvins increase only during the resolution of inflammation (8, 9). Three human lipoxygenase activities have been described in the context of leukotriene and lipoxin synthesis: the 5-lipoxygenase, the 12-lipoxygenase and the 15-lipoxygenase activity. The naming convention for these enzymes is determined by the position of the carbon which is oxidised from the carboxy end of the fatty acid. In general, the pro-inflammatory effect of the products of the 5-lipoxygenase pathway is counteracted by arachidonic acid metabolites generated by the 12- and 15-lipoxygenase pathways (8, 9).



**Figure 1** Biosynthetic pathways and receptors for leukotrienes and lipoxins derived from the omega-6 fatty acid, arachidonic acid. cPLA2, cytosolic phospholipase A2; FLAP, 5-lipoxygenase activating protein; ALOX5, 5-lipoxygenase; ALOX12/15, 12-lipoxygenase/15-lipoxygenase; COX-2, cyclooxygenase-2; LTA4H, leukotriene A4 hydrolase; LTC4S, leukotriene C4 synthase; LTA4, leukotriene A4; LTB4, leukotriene B4; LTC4 – E4, cysteinyl leukotriene C4 – cysteinyl leukotriene E4; 15-S-HETE, 15S-hydroxy-eicosatetraenoic acid; 15-R-HETE, 15R-hydroxy-eicosatetraenoic acid; 15-oxo-EETE, 15-oxo-eicosatetraenoic acid; LXA4, lipoxin A4; 15-epi-LXA4, 15-epi-lipoxin A4 (aspirin-triggered lipoxin A4); BLT1 and BLT2, leukotriene B4 receptor 1 and 2; ALXR, lipoxin A4 receptor (FPRL1); AHR, aryl hydrocarbon receptor; CysLT1 and CysLT2, cysteinyl leukotriene receptor 1 and 2; +, activation of receptor; –, blocking of receptor.

## Biosynthesis of leukotrienes

The initial steps in leukotriene biosynthesis (Figure 1) involve the oxygenation of arachidonic acid by 5-lipoxygenase in the presence of 5-lipoxygenase activating protein (FLAP). During these steps arachidonic acid is oxygenated to LTA4. Then, this labile metabolite is converted by either LTA4 hydrolase (LTA4H) to LTB4 or by LTC4 synthase (LTC4S) to cysteinyl leukotrienes (10). Synthesis of cysteinyl leukotrienes proceeds with conjugation of LTA4 with reduced glutathione by LTC4 synthase to form LTC4. This active cysteinyl leukotriene is then exported from the cells into the extracellular compartment through a specific ABC transporter (11), where it is converted to cysteinyl leukotrienes LTD4 and LTE4 through sequential amino acid hydrolysis.

There is evidence that the formation of leukotrienes, LTB4 and cysteinyl leukotrienes is mediated for some part through transcellular mechanisms involving leukocytes and non-myeloid cells. While the expression of 5-lipoxygenase is restricted to myeloid cells, LTA4H and LTC4S are expressed in several tissues and cell types including erythrocytes, platelets, endothelial cells, eosinophils and mast cells (12–14). Thus, it is thought that a considerable part of the intermediate metabolite LTA4 is secreted from myeloid cells and converted to LTB4 or cysteinyl leukotrienes by non-myeloid cells (15, 16). The separation of the rate limiting enzymes in different cell types may constitute an additional regulatory mechanism to control the production of these important

mediators and contribute to the overall inflammatory response (15, 16).

The synthesis of the leukotrienes is regulated by the amount of arachidonic acid released from phospholipids from the cell membrane by cytosolic phospholipase A2 (cPLA2), by the abundance of the enzymes involved in the leukotriene synthesis pathway, and by the subcellular localization of 5-lipoxygenase (17, 18). In resting cells, 5-lipoxygenase resides in the cytoplasm or the nucleoplasm, upon activation it translocates to the nuclear membrane to interact with FLAP. FLAP has no enzymatic activity, but improves the interaction of 5-lipoxygenase with the substrate and locates the 5-lipoxygenase to the nuclear membrane (19). 5-Lipoxygenase maintains maximal efficiency when it is located at the inner nuclear membrane, leading to maximal LTB4 synthesis (20). Phosphorylation of two serins was shown to regulate the nuclear localization of 5-lipoxygenase, with phosphorylation of Ser-523 inhibiting nuclear import (21) and phosphorylation of Ser-271 inhibiting nuclear export (22).

## Biosynthesis of lipoxins

Lipoxins are increasingly recognized as key mediators in the resolution of inflammation. They are synthesized by three major pathways (Figure 1). The first pathway, initiated by 5-lipoxygenase, was first described during platelet-leukocyte interaction (23). Initially, LTA4 is released by activated leu-

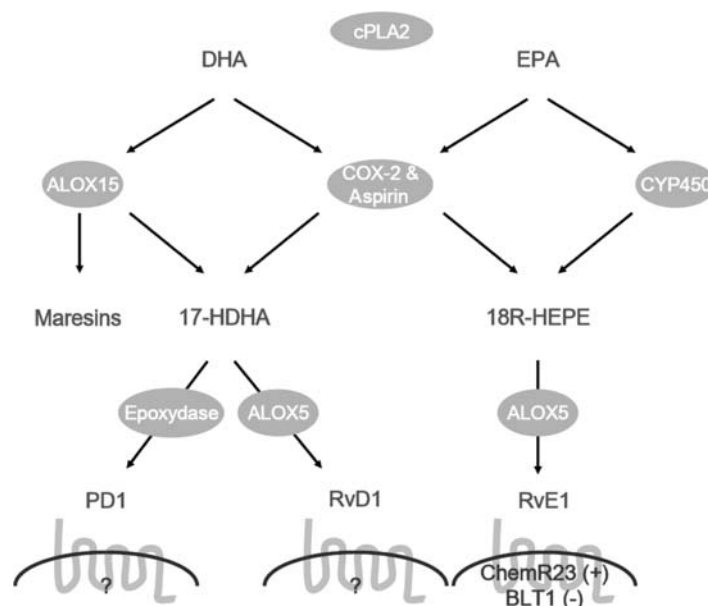
kocytes and then made available for further metabolic processes with neighboring cells (23). When platelets and leukocytes aggregate, the human 12-lipoxygenase converts LTA<sub>4</sub> to lipoxin A<sub>4</sub> and B<sub>4</sub>. Through this interaction, platelets may become the major source of lipoxins, given their high expression of 12-lipoxygenase, although they cannot produce lipoxins on their own (23).

The second pathway is initiated by 15-lipoxygenase activity from ALOX15. The role of this 15-lipoxygenase pathway is most clearly demonstrated in airway epithelial cells, such as monocytes or eosinophils, which upregulate ALOX15 expression following stimulation with IL-4 and IL-13 (24, 25). These two cytokines are negative regulators of inflammation, and are thought to play a role in the resolution of inflammation (26). When the cells are stimulated by these cytokines they generate 15S-hydroxy-eicosatetraenoic acid (15-S-HETE). These compounds are rapidly converted to lipoxins by the 5-lipoxygenase of monocytes and neutrophils (27). These transformations can occur in humans following secretion of 15-S-HETE via transcellular routes, or within the cell of origin (23, 28). Recently, it was shown that ALOX15B may also play a role in innate immunity since it is expressed in human macrophages and is regulated by hypoxia, which leads to increased 15-S-HETE production (29). Importantly, 15-S-HETE itself can activate anti-inflammatory peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) receptors (30, 31) and suppresses LTB<sub>4</sub> induced neutrophil chemotaxis (32). However, its primary anti-inflammatory function may well be as a precursor for the more potent lipoxins.

The third pathway for lipoxin production involves the anti-inflammatory drug acetylsalicylic acid (aspirin), which is known to suppress prostaglandin synthesis through covalent acetylation of the COX-2 enzyme. However, acetylated COX-2 is still enzymatically active and produces 15-R-HETE instead of prostaglandins from arachidonic acid (33). This metabolic intermediate is then converted through 5-lipoxygenase activity to the 15-epi-lipoxins, also called aspirin-triggered lipoxins (ATL). 15-Epi-lipoxins are more potent and longer acting than their native 15S-containing lipoxin forms because they are less rapidly metabolized (34, 35). Biosynthesis of these 15-epi-lipoxins was shown to derive, in part, from transcellular pathways between endothelial cells and leukocytes (33), with the endothelial cells supplying acetylated COX-2 enzyme in the presence of aspirin, and leukocytes adding the 5-lipoxygenase activity (36). Indeed, the formation of 15-epi-lipoxins has been detected *in vivo* in an aspirin-dependent manner in several murine models of inflammation (37), as well as in aspirin-treated healthy volunteers (38, 39).

### Biosynthesis of resolvins and protectins

In contrast to the lipoxins which derive from arachidonic acids, the resolvins and protectins are produced from omega-3 fatty acids (Figure 2) by sequential metabolism of CYP450 or acetylated COX-2 enzyme and lipoxygenases (40, 41). The D-series of resolvins and protectins are derived from omega-3 fatty acid docosahexaenoic acid (DHA), while the



**Figure 2** Biosynthetic pathways and receptors for protectins and resolvins derived from the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

cPLA2, cytosolic phospholipase A2; ALOX15, 15-lipoxygenase; COX-2, cyclooxygenase-2; CYP450, cytochrome P-450 enzyme; ALOX5, 5-lipoxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; 17-HDHA, 17-hydroxy-docosahexaenoic acid; 18R-HEPE, 18R-hydroxy-eicosapentaenoic acid; PD1, protectin D1; RvD1, resolvin D1; RvE1, resolvin E1; ChemR23, chemerin receptor (CMKLR1); BLT1, leukotriene B<sub>4</sub> receptor 1.

E-series are derived from eicosapentaenoic acid (EPA). Two pathways each have been proposed for formation of the resolvins series and for the protectins (Figure 2).

One of the pathways for the D-series involves 5- and 15-lipoxygenase activity (42), with evidence that 15-lipoxygenase activity from leukocytes or epithelial cells leads to 17S-hydroxy-DHA (42, 43). This has anti-inflammatory effects and can be metabolized further by leukocyte 5-lipoxygenase to the D-series of resolvins or by an epoxide hydrolase to the protectins (40, 42, 44). In the second pathway, the intermediate metabolite 17R-hydroxy-DHA is formed from DHA by acetylated COX-2 (40) and further metabolized into the D-series of resolvins and protectins (45). These transformations can occur through transcellular biosynthesis between endothelial cells and leukocytes, or within a single cell type in leukocytes.

Two pathways are also proposed for the generation of resolvins of the E-series from EPA, which involve 5-lipoxygenase activity (Figure 2). Initially, acetylated COX-2 or CYP450 transform EPA to the intermediate metabolite 18R-hydroxy-eicosapentaenoic acid (18R-HEPE). This is then metabolized by the leukocyte 5-lipoxygenase to form the E-series of resolvins (40, 46, 47). Both pathways were shown to be transcellular processes where the intermediate 18R-HEPE was released from aspirin or cytokine treated endothelial cells and subsequently metabolized to resolvin E1 by leukocytes (46, 47).

## Biosynthesis of other lipid mediators

The 5-lipoxygenase pathway is also involved in the production of the five series of leukotrienes (LTB<sub>5</sub>) which derive from EPA instead of arachidonic acid, and which results in the four series of leukotrienes (LTB<sub>4</sub>). The five series of leukotrienes have reduced pro-inflammatory and vasoactive properties compared to the four series leukotrienes and are thought to attenuate inflammation (48). Other lipoxygenase derived metabolites have been described in macrophages like 15-oxo-eicosatetraenoic acid (15-oxo-EETE) and the macrophage mediators in resolving inflammation (maresins), which derive from arachidonic acid and DHA, respectively. Not much is known about the physiological or pharmacological effects of these two classes, but there are indications that 15-oxo-EETE inhibits endothelial cell proliferation (49), and that maresins are potent mediators for resolving inflammation (50). Another class of arachidonic acid derived lipid mediators are the hepoxilins which were shown to inhibit lung fibrosis and inflammation in mice, and to increase circulating insulin concentrations in rats (51).

## Inflammatory effects of leukotrienes

LTB<sub>4</sub> is predominantly generated by neutrophils and monocytes/macrophages, as well as by a subset of mast cells (52). In vivo, LTB<sub>4</sub> is a chemo attractant for leukocytes and increases leukocyte rolling and adhesion to the endothelium,

followed by transendothelial migration. In fact, LTB<sub>4</sub> is among the most potent leukocyte stimuli (53) and also stimulates secretion of superoxide anion and release of different granule constituents from leukocytes (54, 55). These inflammatory effects are mediated through LTB<sub>4</sub> specific G-protein-coupled receptors (Figure 1), the BLT1 and the BLT2 receptors (52).

The cysteinyl leukotrienes are produced by eosinophils, basophils and mast cells, and have been identified as the slow reacting substances of anaphylaxis (56). The cysteinyl leukotrienes are the most potent bronchoconstrictive substances described (57). They result in plasma exudation in all airway segments (58), stimulate mucus secretion in isolated airways (59, 60), increase infiltration of eosinophils into the airway mucosa of patients with asthma (61), and may be involved in smooth muscle proliferation and remodelling in airways (62). Given their high potential as pro-inflammatory and bronchoconstrictive mediators, cysteinyl leukotriene over production during asthma can contribute to formation and maintenance of airway inflammation and airflow obstruction (63). Two receptors belonging to the family of G-protein-coupled receptors (CysLT1 and CysLT2) have been identified which mediate the effects of the cysteinyl leukotrienes (Figure 1). The CysLT1 receptor is stimulated by all CysLTs, while the CysLT2 receptor is stimulated primarily by LTC<sub>4</sub> and LTD<sub>4</sub> (63). Several drugs blocking the CysLT1 receptor have been developed and are now used to treat patients with asthma.

## Anti-inflammatory effects of lipoxins and resolvins

The 12- and 15-lipoxygenase derived lipoxins antagonize the pro-inflammatory effects of leukotrienes, reduce neutrophil chemotaxis and block neutrophil entry across the intestinal epithelium (64). Lipoxin A<sub>4</sub> and B<sub>4</sub> both attenuate leukotriene C<sub>4</sub> and B<sub>4</sub> responses and reduce neutrophil adherence to human umbilical vein endothelial cells (65), which likely results from downregulation of P-selectin expression (66). Application of lipoxins and stable lipoxin analogues in vivo also antagonize VEGF induced endothelial cell proliferation and angiogenesis (67), leukotriene C<sub>4</sub> mediated vasoconstriction in patients with asthma (68), and significantly inhibit both neutrophil infiltration and neutrophil-mediated vascular injury in mice (69). Blocking of leukotriene C<sub>4</sub> signalling was effected by competition of the lipoxins with the leukotrienes for binding sites at the CysLT receptor.

Also, there is a direct anti-inflammatory effect of lipoxins that is independent of leukotrienes and their cognate receptors. Recently, the G-protein-coupled receptor FPRL1 was shown to display specific and selective lipoxin A<sub>4</sub> binding (70). Upon stimulation with lipoxin A<sub>4</sub>, this receptor, now termed lipoxin A<sub>4</sub> receptor (ALXR), transmits signals which result in the regulation of the p38 MAPK cascade and attenuation of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) induced nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation (71, 72). These two pathways are implicated in chemotaxis and pro-inflammatory reactions,



respectively. The second receptor involved in lipoxin signaling is the aryl hydrocarbon receptor (AHR) which was shown to signal lipoxin mediated SOCS-2 expression (73). The SOCS proteins are thought to regulate cytokine triggered signal transduction by docking to the intracellular domains of the receptors, or by facilitating proteasome dependent degradation of transcription factors (74). Thus, they play a major role in resolution of inflammation (74).

The resolvins (Figure 2) have potent anti-inflammatory effects, similar to the lipoxins. However, they appear to exert their effects through a different set of receptors (75). Several studies have shown that certain members of the resolvins family suppress neutrophil infiltration and cytokine secretion in animal models of inflammation (42, 76). Also, they have been shown to be protective following brain injury after experimental stroke (77), for insulin-resistance and hepatic steatosis in obesity (78), airway inflammation (79, 80), and they enhanced wound healing and suppressed colitis (75) in mouse models of inflammatory diseases. Similar to lipoxins, resolvins suppress inflammation by interfering directly with the effect of leukotrienes on the receptor level and by signalling through designated receptors. Resolvin E1 seems to suppress inflammation as a partial agonist for BLT1, dampening the effect of leukotrienes, and by signalling through a specific receptor, ChemR23, which was shown to transmit anti-inflammatory signals (75, 81).

Protectin D1 was originally observed as the first anti-inflammatory metabolite of the omega-3 fatty acid DHA with anti-apoptotic and anti-inflammatory effects (82). Protectin D1 protects human retinal pigment epithelial cells from oxidative stress and ganglion cells from cell death (82–84). Also, it is neuroprotective in models of experimental stroke by down regulating leukocyte infiltration following brain ischemia reperfusion, and reduces infarct size (77). Overall, protectin D1 promotes brain cell survival through the induction of antiapoptotic and neuroprotective gene-expression programs (85).

## Role of the 5-lipoxygenase pathway in atherosclerosis

The initial indications for a role of 5-lipoxygenase in atherosclerosis came from mouse studies which identified a locus on mouse chromosome 6 that confers almost total resistance to atherogenesis (86). Examining the congenic region of the locus for potential positional candidate genes revealed that the 5-lipoxygenase was centered under the linkage peak, and subsequent generation of mice deficient in the 5-lipoxygenase showed the same atherosclerosis resistant phenotype (87). Later, investigations in mice lacking the 5-lipoxygenase could not corroborate this pro atherosclerotic effect, but linked the 5-lipoxygenase pathway to hyperlipidemia dependent inflammation of the arterial wall and to the pathogenesis of aortic aneurysms (88).

The enzymes necessary for leukotriene production are expressed in human atherosclerotic lesions (89), and the expression of these enzymes correlates with disease severity

(90). For example, 5-lipoxygenase, FLAP and the LTA4 hydrolase are localized in macrophages of human lesions and the number of positive cells increases in advanced lesions (89, 90). Also, there is evidence that 5-lipoxygenase and LTA4 hydrolase expression are higher in patients with vulnerable plaques compared with patients with stable lesions, and that this increase is associated with clinical events, such as acute ischemic syndromes (90, 91). The mechanism leading to vulnerable plaques may include an increase in metalloproteinase expression and activity (MMP-2 and MMP-9) mediated by 5-lipoxygenase activity (92). Indeed, 5-lipoxygenase dependent production of LTB4 and signaling through the BLT1 receptor has recently been shown essential for MMP-2 and MMP-9 activation of vascular smooth muscle cells by 4-hydroxynonenal, a product of lipid peroxidation (93). Similarly, LTB4 was shown to increase the intima/media thickness and expression of MMP-2 and MMP-9 in human arteries following balloon dilatation and stent implantation (92).

Another indication for the involvement of leukotrienes in atherosclerosis comes from the finding that the LTB4 receptor is expressed on all human cell types involved in atherosclerosis (94), and that antagonists of the LTB4 receptor reduce formation of lesions in mice (95). Treatment with receptor antagonists resulted in fewer macrophages infiltrating the lesion. Similarly, mice deficient in the LTB4 receptor *BLT1* showed reduced lesions when fed an atherogenic diet for up to eight weeks (96). However, longer treatment resulted in similar lesions in *BLT1* deficient and litter mate control mice. This may be due to the presence of BLT2 receptors on macrophages from *BLT1* deficient mice. Such macrophages still react to a chemotactic gradient of LTB4 (96).

Further support for the involvement of 5-lipoxygenase in atherosclerosis comes from genetic studies in humans. In the first set of studies, the potentially functional SP1 repeat polymorphism in the human 5-lipoxygenase promoter showed an association with the degree of intima-media thickness in the common carotid artery (97), and with coronary artery disease in the ADVANCE cohort (98). However, no association was seen with myocardial infarction (99). In the second set of studies, *FLAP* was identified as a susceptibility gene for myocardial infarction and stroke following genome wide screening performed in Icelandic individuals (100). Initially, two different *FLAP* haplotypes were associated with almost a two-fold increase in risk for myocardial infarction, and a 1.5-fold increase for stroke in different ethnicities (100, 101). While the role of *FLAP* in atherosclerosis may be controversial for some ethnicities, the B haplotype of *FLAP* was associated with an increased risk of coronary artery disease in four independent reports from European populations, substantiating the role of this gene in the pathogenesis of coronary artery disease (98, 102–107). In addition, treatment of carriers with this haplotype using a *FLAP* inhibitor led to a modest reduction in LTB4 and myeloperoxidase production in leukocytes ex vivo in a prospective placebo controlled trial (108). This suggests that *FLAP* inhibition may reduce some oxidative and inflammatory stress, and that such treatment may be beneficial in patients with atherosclerosis. In con-

trast, a recent meta-analysis that included 9760 samples found that neither the A or B haplotype of FLAP are associated with increased risk for stroke. However, two independent polymorphisms that are part of these haplotypes were shown to moderately increase the risk for stroke (109). It should be emphasized that the mechanism of how haplotype B could alter LTB<sub>4</sub> production and promote atherosclerosis is currently unknown. A recent study found no difference in LTB<sub>4</sub> production in neutrophils from carriers with different FLAP haplotypes (110).

Inhibition of 5-lipoxygenase and FLAP will not only lead to reduced LTB<sub>4</sub> synthesis, but will also reduce the levels of the cysteinyl leukotrienes, which may also play a role in atherosclerosis. Support for the role of the cysteinyl leukotrienes in atherosclerosis comes from animal model intervention studies using specific CysLT<sub>1</sub> receptor blockers. These drugs have been developed and are now used in asthma patients to interfere with the pro-inflammatory and bronchoconstrictive effect of the cysteinyl leukotrienes. When the CysLT<sub>1</sub> receptor blocker montelukast was given to mice prone to atherosclerosis, the mice showed decreased atherosclerosis (111). Similarly, montelukast was effective in reducing atherosclerosis in a rabbit model of restenosis, where the extent of the reduction was as effective as atorvastatin treatment (112).

The role of the cysteinyl leukotriene pathway on atherosclerotic disease has also been investigated in human genetic studies using a functional polymorphism in the promoter region of the leukotriene C<sub>4</sub> synthase gene (*LTC<sub>4</sub>S*). This polymorphism was shown to increase leukotriene C<sub>4</sub> synthase activity and LTC<sub>4</sub> production in eosinophils (113), and was associated with aspirin intolerant asthma (114). Investigation of this polymorphism in the large prospective Copenhagen City Heart Study revealed a protective effect of the -444 CC-genotype for risk of ischemic cerebrovascular disease, but not for coronary disease (115, 116). However, the same C-allele was associated with increased coronary calcification and carotid atherosclerosis in women enrolled in the longitudinal Muscatine Study (117). These contradictory results have been explained by the distinct effects of the cysteinyl leukotrienes on several cell types involved in atherosclerosis. It also emphasizes that the role of cysteinyl leukotrienes in human atherosclerosis remains to be elucidated. The fact that the enzymes necessary for the formation of the cysteinyl leukotrienes and the CysLT receptors are expressed in normal and diseased arteries (94) warrants further research on the role of this class of lipid mediators in atherosclerosis.

### Role of the 12- and 15-lipoxygenase pathways in atherosclerosis

There is evidence for an anti-inflammatory effect of 15-lipoxygenase through the generation of lipid mediators involved in the resolution of inflammation (118). The oxidation of arachidonic acid by human 15-lipoxygenases leads preferentially to 15-HETE [for review see (118)]. This can either serve directly as an activator of the PPAR $\gamma$  receptor

(30), or can be further metabolized to lipoxins (119). Because the lipoxins were shown to inhibit chemotaxis, adhesion and transmigration of neutrophils, and to antagonize the pro-inflammatory effects of leukotrienes (120), 15-lipoxygenase is thought to play an anti-inflammatory role in the pathology of chronic inflammatory diseases (121).

There is also evidence for a pro-atherosclerotic effect of 15-lipoxygenase through the formation of oxLDL and its role in angiotensin II mediated mechanisms and vascular smooth muscle cell proliferation (122–126). Hence, 15-lipoxygenases seem to play a dual role, with an anti-inflammatory effect through lipoxin and resolvins production, and a pro-inflammatory and atherogenic effect through oxLDL formation and participation in signaling pathways (118).

Animal models of atherosclerosis have not solved the question of whether 15-lipoxygenase activity is pro- or anti-atherogenic because different animal models have shown contrasting results. Monocyte specific 15-lipoxygenase expression in transgenic rabbits reduced atherosclerosis and supported the anti-inflammatory role of 15-lipoxygenase (127, 128). Similarly, an extensive mouse study that used several overexpressing and knockout mouse lines showed an atheroprotective effect of 15-lipoxygenase (129). However, conditional macrophage-specific and general disruption of the mouse homologue 12-lipoxygenase gene reduced atherosclerosis (130, 131), while overexpression of human 15-lipoxygenase in vascular endothelium enhanced atherosclerosis in other mouse strains (132).

The discrepancies between the different animal models have been explained by the different positional selectivity of mammalian 12- and 15-lipoxygenase iso-enzymes which oxidize arachidonic acid at carbon atoms 12 and 15, and which have different expression patterns (118). Humans have three 12-lipoxygenases and two 15-lipoxygenases which show discrete expression patterns, substrate specificities, and stereo-selective metabolism (118). However, only ALOX12 and two 15-lipoxygenases, ALOX15 and ALOX15B, seem to be expressed in cells involved in atherosclerosis. Another explanation for the discrepancies between the different animal models was recently proposed from results of a study in mice lacking 5-lipoxygenase and 15-lipoxygenase activity, either individually or combined (133). In these experiments, shunting of substrate from the 15-lipoxygenase pathway to the 5-lipoxygenase pathway was observed following inactivation of the 15-lipoxygenase pathway. Such intra- and even intercellular shunting of substrate seems to be common (134), and may lead to ambiguous findings depending on the genetic background, immune status of the animals, and on the composition of the food used.

These different effects of 15-lipoxygenase on atherosclerosis in different model systems preclude a prediction of the role of 15-lipoxygenase in human atherosclerotic disease. To answer this question, genetic studies have been performed in humans to investigate the association of the human 15-lipoxygenase with coronary artery disease and myocardial infarction. Two rare functional polymorphisms have been characterized in the 15-lipoxygenase gene which lead to increased (c.-292C>T) (135) and reduced (T560M) (136)

enzyme activity. While the activating c.-292C>T polymorphism showed a trend for an atheroprotective effect in a small case control study of coronary artery disease, the inactivating T560M polymorphism was associated with a significantly increased risk for coronary artery disease in the ADVANCE study (136, 137). These results indicate that ALOX15 may be anti-inflammatory and anti-atherogenic in humans. However, corroboration of such an atheroprotective effect of the 15-lipoxygenase gene was unsuccessful in a recent large case control study, the MONIKA/KORA cohort which investigated the effect of these polymorphisms on myocardial infarction (138). In this study, the activating c.-292C>T polymorphism showed no effect on the risk for myocardial infarction, while the inactivating T560M polymorphism showed a trend for an association with myocardial infarction, with a similar risk increase (OR 1.7) as reported in the ADVANCE study (136). Thus far, all large study samples from Caucasians investigating the association of the inactivating polymorphism (T560M) in 15-lipoxygenase showed a similar risk increase. However, this was found not to be significant in two of the studies because of the low frequency of the T560M polymorphism (136, 138). Taken together, the role of the 15-lipoxygenase enzyme in human atherosclerosis has not yet been solved, although there is currently more support for a neutral or atheroprotective role for 15-lipoxygenase in human disease, than for promoting atherosclerosis.

## Conclusions and perspectives

The lipoxygenase pathways play a role in leukocyte recruitment and activation, with the generation of leukotrienes, lipoxins and resolvins. Although most of the information on these mediators derives from animal models of acute inflammation or asthma, there is evidence that they also play a role in human chronic inflammatory diseases, such as rheumatoid arthritis and atherosclerosis. The fact that the enzymes necessary for the production of the pro-inflammatory leukotrienes are expressed in human atherosclerotic lesions, and that the expression of these enzymes seems to correlate with disease severity and features of vulnerable plaque, makes the 5-lipoxygenase pathway and the receptors for the generated leukotrienes interesting targets for drug intervention in human atherosclerosis. Such anti-leukotriene agents which have been developed to treat patients with asthma are now being investigated for their effectiveness in the cardiovascular setting using intermediate cardiovascular endpoints. In addition to these currently used 5-lipoxygenase inhibitors and CysLT receptor blockers, there are novel drugs emerging which block the BLT1 receptor or which inhibit several metabolizing enzymes together; i.e., COX-2 and 5-lipoxygenase. Intervention studies with these drugs will further show the role of the 5-lipoxygenase pathway in human atherosclerosis, and may lead to a novel class of drugs to slow the disease.

The role of 15-lipoxygenase in human atherosclerosis is not yet clear, and human research is necessary to clarify this

role. To this end, large human genetic studies investigating the association of the two rare functional polymorphisms with different clinical end points may delineate the role of the enzyme in human atherosclerotic disease. However, less ambiguity exists about the effect of the lipid mediators produced by the 15-lipoxygenase pathway, the lipoxins and the resolvins. These lipid mediators have anti-inflammatory and pro-resolution properties. Also, the stable forms of these mediators may have the potential to serve as a novel class of anti-inflammatory drugs. However, the usefulness of these drugs to decrease atherosclerosis will depend on the expression of the specific receptors for these lipid mediators on cells present in the atherosclerotic plaque, and on their effect on chemotaxis and activation of leukocytes. It will be essential to investigate whether increased monocyte recruitment by some of these anti-inflammatory lipid mediators will be protective, or accelerate atherosclerosis.

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